

chondria, are usually present near gap junctions but not near simple appositions. The fact that the two distinct types of junctions occur within the same blood vessel, or in two different kinds of smooth muscle within the same tissue sections, argues against the possibility that they represent the same type of contact which has been altered by conditions of fixation. Presumably, simple

appositions, the only contacts present in terminal arterioles, are involved in cell-to-cell propagation of activity¹⁰.

Zusammenfassung. Nachweis, dass in Arteriolen sowie in präkapillaren Arteriolen des Hunde-Duodenums nur eine einfache Aneinanderlagerung der Muskelzellen erfolgt und keine «gap junctions», wie sie in den Muskelschichten der Hohlwandorgane vorhanden sind.

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Septate-Like Junctions Between Spermatogonia in Human Seminiferous Epithelium

Since the first description of septate desmosomes between adjacent epithelial cells of the Hydra by WOOD¹, septate junctions have been found between various epithelial cells of invertebrates²⁻¹¹. From studies on sections tangential to the plasma membrane, it has become evident that the septa form a well defined hexagonal network. The functional significance of these junctions is not fully understood. They were initially thought to be the only sites of low electrical resistance^{12,13}. But recent reports have indicated that macular gap junctions, which have been implicated in low resistance coupling of vertebrate tissues, can also be found along with septate junctions in invertebrate epithelia^{8-10,14-16}.

Although septate junctions were originally thought to be a unique feature of invertebrate epithelial cells, they have now been reported in several vertebrate tissues. LASANSKY et al.¹⁷ found them at synaptic endings of the turtle visual cells, GOBEL¹⁸ in the basket formation of the cat cerebellar cortex, and SOTELO and LLINAS¹⁹ in the cerebellar cortex of rats and cats. The morphological features of the junctions between neuronal elements of vertebrates closely resemble those described for invertebrates. Yet, there are some differences with regard to their length and frequency of occurrence. The well documented septate junctions between cells of the rat adrenal cortex reported by FRIEND and GILULA²⁰ seem to

be real structures, although they differ from the septate desmosomes of invertebrates with regard to periodicity and structure of the septa.

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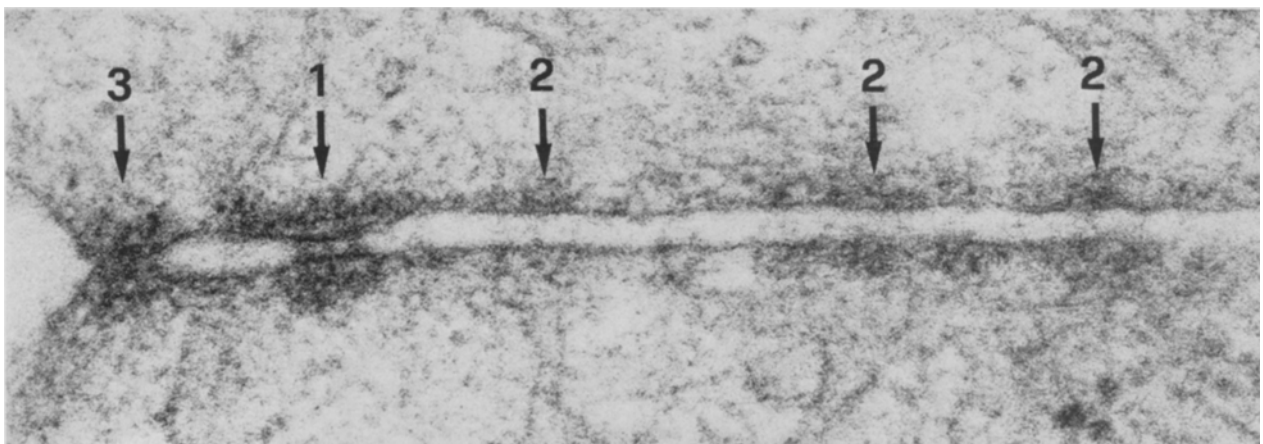


Fig. 1. Electron micrograph showing a contact area between 2 type A spermatogonia in a patient with impaired fertility. At 1 the intercellular space is narrowed and bridged by 3 septa. The apposed plasma membranes are densified, and subjacent to them condensations of cytoplasmic material are evident. The numbers 2 indicate other accumulations of cytoplasmic material and vague intercellular structures. At 3 an undetermined contact area is seen. $\times 150,000$.

During our ultrastructural investigation on cell contacts in human seminiferous epithelium, septate junctions between spermatogonia were observed in two cases, which are reported in this paper.

Material and methods. Testicular biopsies from a 27-year-old patient with impaired fertility and varicocele, and normal testicular tissue from a 34-year-old car accident victim, excised immediately after death, were studied. The biopsy material was fixed by immersion in 2% glutaraldehyde buffered at pH 7.4 with 0.1 M sodium cacodylate. Normal testicular tissue was fixed by vascular perfusion with a solution containing 2.5% glutaraldehyde and 2.5% formaldehyde buffered at pH 7.4 with 0.1 M phosphate (for details see reference²¹). Thin sections were examined with a Philips EM 201 microscope.

Observations. In the biopsy material from the patient with impaired fertility, septate junctions are occasionally found between type A spermatogonia. In areas where the apposed plasma membranes run straight and parallel to each other in a distance of 22 nm, a narrowing of the intercellular space down to 12.5 nm over a short distance

can be observed. The narrowed intercellular gap is regularly bridged by a short series of parallel lamellae which are about 15 nm thick. The opacity of the plasma membranes is greater than in the neighbouring regions, and subjacent to them an accumulation of cytoplasmic material becomes evident. Condensations of cytoplasmic material under parallel arranged plasma membranes can also be found in areas where the intercellular space is not narrowed, but where vague intercellular structures suggest incomplete septa (Figure 1). Such features could reflect sites of new formation of septate junctions³.

Very rarely, short septate junctions are also observed between type A or B spermatogonia of the normal testicular tissue. But here the morphological features are not as uniform as those described in the first case. The width of the intercellular space varies remarkably from one junction to another (12 to 25 nm), and there is no condensation of cytoplasmic material subjacent to the

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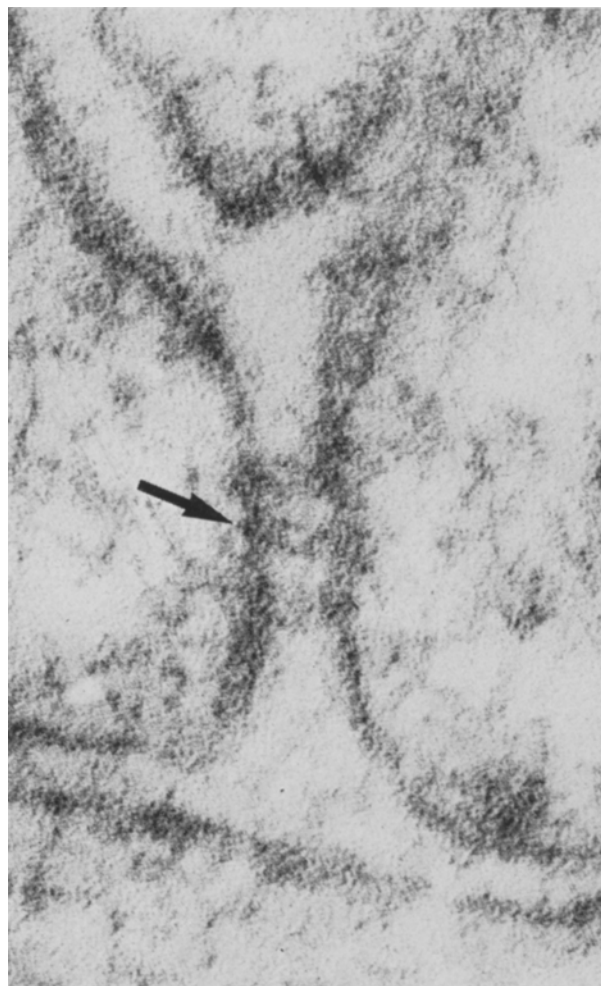


Fig. 2. A septate junction between 2 type A spermatogonia in normal testicular tissue. The intercellular gap is normally wide, and there is no condensation of cytoplasmic material. The arrow points to a septum with 2 denser outer lines. $\times 246,000$.



Fig. 3. More oblique section of a septate junction between 2 type B spermatogonia in normal testicular tissue. The septa appear as bars spanning the narrowed intercellular gap as well as the apposed plasma membranes. In the lower part of the micrograph, the intercellular space is irregularly dilated; in the upper part the plasma membranes are blurred, indicating a possible membrane alteration. $\times 168,000$.

plasma membranes. But again the septa are about 15 to 17 nm thick, arranged with regular periodicity (Figures 2 and 3). In sections normal to the cell surface, the septa sometimes show two outer lines of higher density, as described in invertebrate septate junctions (Figure 2). In more oblique sections, the septa seem to cross not only the intercellular gap but also the apposed plasma membranes (Figure 3).

The septate junctions most often occur between two areas where the intercellular space is irregularly dilated. The plasma organelles and the nucleus of the involved spermatogonia, however, reveal no pathological phenomena. As the longest junction could be followed only over 500 nm and appeared only in few serial sections, these cell contacts possibly represent macular structures and do not surround the cells completely²². Since we failed to obtain tangential sections of these junctions, no conclusions regarding the spacial configuration of the septa can be made.

Discussion. The septate junctions between human spermatogonia differ noticeably from the well defined invertebrate septate junctions: The septa are more than twice as thick, and the periodicity about three-fold greater than in invertebrate septate junctions. Moreover, the contact areas are very short and extremely rare. On the other hand, the periodicity is very regular, and the structural characteristics in the two patients are very similar.

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²⁷ The authors wish to express their sincere thanks to Miss L. KLÄUSLI for her competent technical assistance and to Miss M. MICHEL for her help in preparing the manuscript.

BULGER and TRUMP²³ emphasize that septate junctions in renal tubular epithelium of the rat and the English sole appeared only under certain experimental conditions. Likewise, YAMAMOTO and KONDO²⁴ interpret the septate junctions observed between adjacent mitochondria of rats and cats as a consequence of exceptional circumstances. Our material is too small to determine whether the septate junctions between human spermatogonia are real structures or artefacts. The often irregularly dilated intercellular space around the contact areas could indicate a certain artificial membrane alteration.

An interesting observation has recently been reported by GORIUS et al.²⁵ and FLANDRIN et al.²⁶ who found septate junctions between erythroblasts in a case of refractory anemia. Considering that septate junctions are a common feature between epithelial cells of invertebrates, i.e. between less differentiated cells, one could speculate that in higher organisms undifferentiated cells such as erythroblasts and spermatogonia under certain conditions could retain the potency to form such contact areas.

Zusammenfassung. Kurze, septiert aussehende Zellverbindungen mit regelmässiger Periodizität der Septen konnten vereinzelt zwischen Spermatogonien bei einem Patienten mit Fertilitätsstörung und in normalem Hodengewebe nachgewiesen werden. Die morphologische Struktur dieser Verbindungen wird mit den septierten Zellverbindungen der Invertebraten verglichen, und einige Beziehungen zu septierten Zellverbindungen bei Vertebraten werden diskutiert.

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Cytology of Experimental Teratomas and Teratocarcinomas

Experimental teratomas and teratocarcinomas can easily be produced in mice by transplanting gastrulation embryos to extrauterine sites^{1,2}. Approximately 50% of such grafts give rise to malignant tumors (teratocarcinomas) and the remaining 50% form benign tumors with a limited growth potential (teratomas)². The present study sought to determine whether benign tumors could be differentiated from malignant tumors in cytologic smears from aspirates obtained with a thin needle.

Material and methods. Teratomas and teratocarcinomas were produced in C3H/H mice by transplanting 7-day-old mouse egg-cylinders under the kidney capsule of adult isogenic hosts². Two months after transplantation 10 tumor-bearing animals were sacrificed and aspiration with a thin needle (0.6 mm in diameter) attached to a syringe with a tight fitting piston was done from solid areas of tumors. The aspirated material was spread on a slide to form a thin film, air dried and stained with May-Grünwald-Giemsa (MGG) or with hemalaun and eosin. Histologic slides were made from all the tumors examined. In addition to the embryo-derived tumors, we biopsied and studied retransplantable neurogenic teratocarcinomas obtained after sequential retransplantation

of an embryo-derived teratoma described in detail previously³.

Results. 5 of the embryo-derived tumors were classified histologically as malignant and 5 as benign. The basis for the distinction was the presence or absence of undifferentiated embryonic stem cells, designated, in accordance with the concept of PIERCE⁴, 'embryonal carcinoma cells'. These cells are the only 'malignant', i.e. rapidly proliferating, element in teratocarcinomas and are not found in benign teratomas. Cytologically it was not possible to differentiate malignant from benign tumors. Aspirates of all 10 tumors contained cells in a continuous spectrum from fully differentiated to undifferentiated. It was possible to identify squamous cells, columnar cells with

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